

neb-183-cip.txt

## SEQUENCE LISTING

<110> Xu, Shuang-yong  
Kobbe, Daniela  
Zhu, Zhenyu  
Samuelson, James

## <120> Methods for Altering the Cleavage Specificity of a Type II Restriction Endonuclease

<130> NEB-183-CIP

<150> 10/150,028  
<151> 2002-05-17

<150> 09/693,146  
<151> 2000-07-02

<160> 26

<170> PatentIn version 3.2

<210> 1  
<211> 1650  
<212> DNA  
<213> *Bacillus pumilus*

<220>  
<221> CDS  
<222> (1)..(1650)

<400> 1	atg aat caa tta att gaa aat gtt aat cta caa aaa tta agg ggt ggg	48
Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly		
1 5 10 15		
tat tac acc cct aaa gtt att gct gac ttt tta tgt caa tgg agt att	96	
Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile		
20 25 30		
caa gat gac aca aag agt gta ctt gaa ccc agt tgt gga gat ggt aat	144	
Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn		
35 40 45		
ttt att gaa tcg gca ata ctt agg ttc aaa gaa ctt agt ata gat aat	192	
Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn		
50 55 60		
gaa caa ctt aaa gga aga att aca gga gta gag cta att gaa gaa gaa	240	
Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu		
65 70 75 80		
gct ttg aaa gtt caa aat cga gca aat gag ttg ggg gtt gat aaa aac	288	
Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn		
85 90 95		

ccu ata gta uat ayc gac tcc ttt caa ttt gca uaa gac dat aug dat  
 Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn  
 100 105 110

aaa aaa ttt gat act att att ggt aat cca cca ttc ata aga tac caa 384  
Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln  
Page 1

## neb-183-cip.txt

115	120	125	
aac ttt cct gaa gag cat cgt agt ata gcc atg gaa atg atg gag gaa Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu 130 135 140			432
cta ggt tta aaa cct aat aaa ctt aca aat atc tgg gtt cca ttt cta Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu 145 150 155 160			480
gtg gta tct gct aca tta ctt aat gaa caa gga aag atg gct atg gtt Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val 165 170 175			528
ata ccg gct gaa tta ttt cag gta aag tat gca gca gaa aca aga att Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile 180 185 190			576
ttt tta tca aag ttt ttc gat cgt atc act ata att aca ttt gaa aaa Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys 195 200 205			624
ctt gtt ttt gaa aat atc caa cag gaa gtt ata cta ctt ctt tgt gaa Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu 210 215 220			672
aag aaa gtt aat aaa ggt aaa gga att cgg gtt att gaa tgc gag aac Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn 225 230 235 240			720
tta gat gga tta aat tcc att gat ttt gta gct ata aat ggt tca aat Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn 245 250 255			768
gtt aaa cct att gaa cac cgt act gaa aag tgg aca aag tat ttc tta Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu 260 265 270			816
aac gaa gat gaa ata ctt ctt tta cag agt tta aag gaa gac aaa cgc Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg 275 280 285			864
gtt aaa aat tgt aat gac tat ttt aag aca gaa gtt ggc tta gtt act Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr 290 295 300			912
gga cga aac gaa ttc ttt atg atg aaa gaa aac caa gta aaa gaa tgg Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp 305 310 315 320			960
aat cta gaa gaa tat aca ata cct gtt aca ggt agg tcc aat cag tta Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu 325 330 335			1008
aaa ggt ata aca ttt aca gaa aat gat ttt cat gaa aat tca atg gaa Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu 340 345 350			1056
caa aag gca att cac cta ttt ttg cca cca gat gaa gat ttt gaa aag Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys 355 360 365			1104

neb-183-cip.txt

tta ccg att gag tgt caa aat tat atc aag tat ggg gaa gaa aaa ggc Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly 370 375 380	1152
ttc cat caa ggc tat aaa acc aga att aga aaa cgt tgg tat ata act Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr 385 390 395 400	1200
cca tct aga tgg gtt cca gat gct ttt gct tta aga cag gtt gat ggc Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly 405 410 415	1248
tat cca aaa cta att tta aat gaa acc gac gct tct tct act gat aca Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr 420 425 430	1296
att cat agg gtt aga ttt aaa gaa ggt ata aat gaa aag tta gcc gta Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val 435 440 445	1344
gtt tca ttt ttg aac tca ctc act ttt gca tct tca gaa ata acg ggg Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly 450 455 460	1392
aga agt tat ggt ggt gtt atg aca ttc gaa cca act gaa att gga Arg Ser Tyr Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly 465 470 475 480	1440
gaa atc cta ata cct tcc ttt gat aac tta tcc att gat ttt gat aaa Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys 485 490 495	1488
att gat gcc tta att cga gaa aag gag att gaa aaa gtc ctt gat att Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile 500 505 510	1536
gtt gat gaa gct tta ctt ata aaa tat cat ggg ttt agt gag aaa gaa Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu 515 520 525	1584
gta aaa cag ctt cga ggg ata tgg aag aaa ctt tct cag aga aga aac Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn 530 535 540	1632
aat aga acg aag aaa taa Asn Arg Thr Lys Lys 545 550	1650

<210> 2  
<211> 549  
<212> PRT  
<213> Bacillus pumilus

<400> 2  
Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly  
1 5 10 15  
Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile  
20 25 30  
Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn

## neb-183-cip.txt

35

40

45

Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn  
 50 55 60

Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu  
 65 70 75 80

Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn  
 85 90 95

Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn  
 100 105 110

Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln  
 115 120 125

Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu  
 130 135 140

Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu  
 145 150 155 160

Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val  
 165 170 175

Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile  
 180 185 190

Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys  
 195 200 205

Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu  
 210 215 220

Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn  
 225 230 235 240

Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn  
 245 250 255

Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu  
 260 265 270

Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg  
 275 280 285

Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr  
 290 295 300

Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp  
 305 310 315 320

Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu  
 325 330 335

Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu  
 340 345 350

Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys  
 355 360 365

neb-183-cip.txt

Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly  
370 375 380

Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr  
385 390 395 400

Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly  
405 410 415

Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr  
420 425 430

Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val  
435 440 445

Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly  
450 455 460

Arg Ser Tyr Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly  
465 470 475 480

Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys  
485 490 495

Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile  
500 505 510

Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu  
515 520 525

Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn  
530 535 540

Asn Arg Thr Lys Lys  
545

<210> 3  
<211> 3030  
<212> DNA  
<213> *Bacillus pumilus*

<220>  
<221> CDS  
<222> (1)..(3030)

<400> 3  
atg cat ata agt gag tta gta gat aaa tac aaa gcg cat aga agt act 48  
Met His Ile Ser Glu Leu Val Asp Lys Tyr Lys Ala His Arg Ser Thr  
1 5 10 15

ttt tta aaa cca act tat aat gaa act caa cta agg aat gat ttt ata 96  
Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile  
20 25 30

gac cca ctt cta aaa tct tta gga tgg gat gtt gat aat acc aaa gga 144  
Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly  
35 40 45

aaa aca cat att cta aga gat gtc att caa gaa gaa tac ata gaa ata 192

## neb-183-cip.txt

Lys	Thr	His	Ile	Leu	Arg	Asp	Val	Ile	Gln	Glu	Glu	Tyr	Ile	Glu	Ile	
50				55					60							
aaa	gat	gag	gag	aca	aag	aaa	aat	cca	gat	tat	aca	ctt	cgt	ata	aac	240
Lys	Asp	Glu	Glu	Thr	Lys	Lys	Asn	Pro	Asp	Tyr	Thr	Leu	Arg	Ile	Asn	
65		70						75							80	
ggt	acg	aga	aag	ctg	ttt	gta	gag	gtt	aag	aaa	ccg	tct	ttt	aat	att	288
Gly	Thr	Arg	Lys	Leu	Phe	Val	Glu	Val	Lys	Lys	Pro	Ser	Phe	Asn	Ile	
				85			90						95			
ttg	aaa	tca	gct	aaa	gca	gcc	ttc	caa	aca	aga	aga	tat	ggt	tgg	agt	336
Leu	Lys	Ser	Ala	Lys	Ala	Ala	Phe	Gln	Thr	Arg	Arg	Tyr	Gly	Trp	Ser	
			100				105					110				
gct	aac	ctt	ggt	att	tca	gta	ctt	aca	aat	ttc	gag	cat	cta	gtt	att	384
Ala	Asn	Leu	Gly	Ile	Ser	Val	Leu	Thr	Asn	Phe	Glu	His	Leu	Val	Ile	
			115				120				125					
tat	gat	tgt	aga	tat	acg	cct	gac	aaa	tcc	gac	aat	gaa	cat	att	gct	432
Tyr	Asp	Cys	Arg	Tyr	Thr	Pro	Asp	Lys	Ser	Asp	Asn	Glu	His	Ile	Ala	
			130			135				140						
aga	tat	aaa	gtt	ttc	tct	tac	gag	gaa	tat	gaa	gaa	gca	ttt	gat	gaa	480
Arg	Tyr	Lys	Val	Phe	Ser	Tyr	Glu	Glu	Tyr	Glu	Glu	Ala	Phe	Asp	Glu	
			145			150			155				160			
ata	aag	gat	ata	att	tca	tat	gag	tca	gcc	aac	tca	ggt	gct	ctg	gac	528
Ile	Lys	Asp	Ile	Ile	Ser	Tyr	Glu	Ser	Ala	Asn	Ser	Gly	Ala	Leu	Asp	
			165				170				175					
gaa	atg	ttt	gat	gta	aat	aca	aga	gtt	ggt	gaa	acg	ttt	gac	gag	tat	576
Glu	Met	Phe	Asp	Val	Asn	Thr	Arg	Val	Gly	Glu	Thr	Phe	Asp	Glu	Tyr	
			180			185				190						
ttt	tta	cag	caa	att	gag	aat	tgg	cgc	gaa	aag	cta	gct	aaa	act	gca	624
Phe	Leu	Gln	Gln	Ile	Glu	Asn	Trp	Arg	Glu	Lys	Leu	Ala	Lys	Thr	Ala	
			195			200				205						
att	aaa	aat	aac	acc	gaa	tta	ggt	gaa	gag	gac	gtc	aat	ttt	att	gtc	672
Ile	Lys	Asn	Asn	Thr	Glu	Leu	Gly	Glu	Glu	Asp	Val	Asn	Phe	Ile	Val	
			210			215				220						
caa	aga	cta	tta	aac	aga	att	att	ttt	ctt	aga	gtt	tgt	gaa	gat	aga	720
Gln	Arg	Leu	Leu	Asn	Arg	Ile	Ile	Phe	Leu	Arg	Val	Cys	Glu	Asp	Arg	
			225			230			235				240			
acc	att	gaa	aaa	tat	gaa	aca	att	aaa	agt	ata	aaa	aac	tat	gag	gaa	768
Thr	Ile	Glu	Lys	Tyr	Glu	Thr	Ile	Lys	Ser	Ile	Lys	Asn	Tyr	Glu	Glu	
			245			250			255				255			
tta	aaa	gat	ctg	ttt	caa	aag	tct	gat	agg	aaa	ttt	aat	tca	ggt	ctc	816
Leu	Lys	Asp	Leu	Phe	Gln	Lys	Ser	Asp	Arg	Lys	Phe	Asn	Ser	Gly	Leu	
			260			265			270							
ttt	gac	ttc	ata	gat	gat	acg	ctc	ttg	ctt	gag	gtt	gaa	att	gat	tcg	864
Phe	Asp	Phe	Ile	Asp	Asp	Thr	Leu	Leu	Leu	Glu	Val	Glu	Ile	Asp	Ser	
			275			280			285							
aat	gta	ttg	ata	gaa	att	ttt	agt	gat	tta	tat	ttc	cca	caa	agc	cca	912
Asn	Val	Leu	Ile	Glu	Ile	Phe	Ser	Asp	Leu	Tyr	Phe	Pro	Gln	Ser	Pro	
			290			295			300							

neb-183-cip.txt

tat gat ttt tct gtt gtc gat cca aca ata tta agc cag ata tat gaa Tyr Asp Phe Ser Val Val Asp Pro Thr Ile Leu Ser Gln Ile Tyr Glu 305 310 315 320	960
cgt ttt cta ggt caa gaa ata att ata gag tca ggt ggt aca ttt cac Arg Phe Leu Gly Gln Glu Ile Ile Glu Ser Gly Gly Thr Phe His 325 330 335	1008
att acg gag tca cca gaa gtt gcg gcg tcc aat ggt gtt gtt cca act Ile Thr Glu Ser Pro Glu Val Ala Ala Ser Asn Gly Val Val Pro Thr 340 345 350	1056
cca aaa att atc gtc gaa cag ata gtg aaa gac act tta acg ccc ctt Pro Lys Ile Ile Val Glu Gln Ile Val Lys Asp Thr Leu Thr Pro Leu 355 360 365	1104
acg gaa ggc aaa aaa ttt aat gag cta tgt aac tta aaa ata gca gat Thr Glu Gly Lys Lys Phe Asn Glu Leu Cys Asn Leu Lys Ile Ala Asp 370 375 380	1152
ata tgt tgt gga tca gga act ttc cta att tca agt tat gac ttt cta Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Asp Phe Leu 385 390 395 400	1200
gta gag aaa gta atg gaa aag ata ata gaa gag aac atc gat gat tca Val Glu Lys Val Met Glu Lys Ile Ile Glu Glu Asn Ile Asp Asp Ser 405 410 415	1248
gat tta gta tat gaa act gaa gaa ggg cta att ttg aca ctt aaa gca Asp Leu Val Tyr Glu Thr Glu Glu Gly Leu Ile Leu Thr Leu Lys Ala 420 425 430	1296
aaa aga aat atc ttg gag aat aat ttg ttt ggt gtt gat gtt aat cca Lys Arg Asn Ile Leu Glu Asn Asn Leu Phe Gly Val Asp Val Asn Pro 435 440 445	1344
tac gct gtt gaa gta gct gag ttc agt tta tta tta aag cta tta gaa Tyr Ala Val Glu Val Ala Glu Phe Ser Leu Leu Leu Lys Leu Leu Glu 450 455 460	1392
ggt gag aat gag gca tcg gtt aat aat ttc att cac gag cat gag gat Gly Glu Asn Glu Ala Ser Val Asn Asn Phe Ile His Glu His Glu Asp 465 470 475 480	1440
aaa ata tta ccg gat tta aca tct att att aaa tgt gga aac agc tta Lys Ile Leu Pro Asp Leu Thr Ser Ile Ile Lys Cys Gly Asn Ser Leu 485 490 495	1488
gta gat aat aag ttt ttt gaa ttc atg cca gaa tcg tta gag gac gat Val Asp Asn Lys Phe Phe Glu Phe Met Pro Glu Ser Leu Glu Asp Asp 500 505 510	1536
gaa atc tta ttt aag gct aat cca ttt gaa tgg gaa gag gag ttt cca Glu Ile Leu Phe Lys Ala Asn Pro Phe Glu Trp Glu Glu Glu Phe Pro 515 520 525	1584
gat att atg gca aat ggt ggc ttt gat gct att ata gga aat cca cct Asp Ile Met Ala Asn Gly Gly Phe Asp Ala Ile Ile Gly Asn Pro Pro 530 535 540	1632
tat gtt cga ata cag aac atg aaa aaa tat agt cct gag gaa att gaa Tyr Val Arg Ile Gln Asn Met Lys Lys Tyr Ser Pro Glu Glu Ile Glu	1680

## neb-183-cip.txt

545	550	555	560	
tat tat caa tca aaa gac tct gaa tat act gtt gca aaa aaa gaa aca Tyr Tyr Gln Ser Lys Asp Ser Glu Tyr Thr Val Ala Lys Lys Glu Thr 565 570 575				1728
gtt gac aag tat ttt tta ttt att gag aga gca tta ata tta ctc aat Val Asp Lys Tyr Phe Leu Phe Ile Glu Arg Ala Leu Ile Leu Leu Asn 580 585 590				1776
cct act ggg ctg ttg ggt tat ata ata ccg cat aaa ttc ttt att aca Pro Thr Gly Leu Leu Gly Tyr Ile Ile Pro His Lys Phe Phe Ile Thr 595 600 605				1824
aaa ggt ggt aag gaa cta aga aag ttc ata gct gaa aaa cat caa ata Lys Gly Lys Glu Leu Arg Lys Phe Ile Ala Glu Lys His Gln Ile 610 615 620				1872
tca aaa att ata aat ttt ggt gtt aca cag gtc ttt cca gga aga gcg Ser Lys Ile Ile Asn Phe Gly Val Thr Gln Val Phe Pro Gly Arg Ala 625 630 635 640				1920
aca tat acg gct att tta att atc caa gca aat aaa atg gca cag ttc Thr Tyr Thr Ala Ile Leu Ile Gln Ala Asn Lys Met Ala Gln Phe 645 650 655				1968
aag tat aag aaa gta agt aat ata tca gca gaa acc cta gat tct gaa Lys Tyr Lys Lys Val Ser Asn Ile Ser Ala Glu Thr Leu Asp Ser Glu 660 665 670				2016
gaa aat acg tgt gtt tat agc tca gaa aag tat aat tct gac cct tgg Glu Asn Thr Cys Val Tyr Ser Ser Glu Lys Tyr Asn Ser Asp Pro Trp 675 680 685				2064
ata ttt tta tct cct gaa aca gaa gct gtt ttt act aaa ttt aca gaa Ile Phe Leu Ser Pro Glu Thr Glu Ala Val Phe Thr Lys Phe Thr Glu 690 695 700				2112
gct caa ttt gag aaa ctt gga gaa atc act gat ata agt gta gga cta Ala Gln Phe Glu Lys Leu Gly Glu Ile Thr Asp Ile Ser Val Gly Leu 705 710 715 720				2160
caa aca agc gct gat aaa ata tat att ttt att cct gaa aat gaa act Gln Thr Ser Ala Asp Lys Ile Tyr Ile Phe Ile Pro Glu Asn Glu Thr 725 730 735				2208
tca gat aca tat ata ttt aat tat aaa ggg aaa aga tat gaa ata gaa Ser Asp Thr Tyr Ile Phe Asn Tyr Lys Gly Lys Arg Tyr Glu Ile Glu 740 745 750				2256
aaa tct ata tgt tgc cca gct atc tat gac tta tct ttt ggt tct ttt Lys Ser Ile Cys Cys Pro Ala Ile Tyr Asp Leu Ser Phe Gly Ser Phe 755 760 765				2304
gaa agc att cag gga aat gca caa atg ata ttc cct tat gaa atc aga Glu Ser Ile Gln Gly Asn Ala Gln Met Ile Phe Pro Tyr Glu Ile Arg 770 775 780				2352
gat gaa gaa gca tat cta cta gag gaa gaa acg ctt gaa aat gat tat Asp Glu Glu Ala Tyr Leu Leu Glu Glu Thr Leu Glu Asn Asp Tyr 785 790 795 800				2400

neb-183-cip.txt

cct ctt gct tgg aat tat ttg aat gag ttt aaa gaa gct ctt gaa aaa Pro Leu Ala Trp Asn Tyr Leu Asn Glu Phe Lys Glu Ala Leu Glu Lys 805 810 815	2448
aga agc tta caa ggc cgt aat ccg aaa tgg tat caa tat ggt cggtcc Arg Ser Leu Gln Gly Arg Asn Pro Lys Trp Tyr Gln Tyr Gly Arg Ser 820 825 830	2496
caa agt tta tca aaa ttt cat gat aaa gaa aaa ctg ata tgg acc gta Gln Ser Leu Ser Lys Phe His Asp Lys Glu Lys Leu Ile Trp Thr Val 835 840 845	2544
ctt gct acg aaa ccc ccg tat gta ctt gat agg aat aac ctg tta ttt Leu Ala Thr Lys Pro Pro Tyr Val Leu Asp Arg Asn Asn Leu Leu Phe 850 855 860	2592
act ggt ggt gga aac gga ccg tat tat ggt tta att aac caa tct att Thr Gly Gly Gly Asn Gly Pro Tyr Tyr Gly Leu Ile Asn Gln Ser Ile 865 870 875 880	2640
tac tct ttg cat tat ttt tta ggt att ctt tca cat cct gta ata gaa Tyr Ser Leu His Tyr Phe Leu Gly Ile Leu Ser His Pro Val Ile Glu 885 890 895	2688
agt atg gta aaa gca agg gcc agt gaa ttt agg gga tca tat tat tct Ser Met Val Lys Ala Arg Ala Ser Glu Phe Arg Gly Ser Tyr Tyr Ser 900 905 910	2736
cat gga aaa caa ttt att gag aaa atc cca att aga aag att gat ttt His Gly Lys Gln Phe Ile Glu Lys Ile Pro Ile Arg Lys Ile Asp Phe 915 920 925	2784
gat gat caa gat gag gta gac aaa tat aat acg gtg gtc aca aca gta Asp Asp Gln Asp Glu Val Asp Lys Tyr Asn Thr Val Val Thr Thr Val 930 935 940	2832
gaa aaa tta att ata act acc gat aga att aaa agt gag agc aat gga Glu Lys Leu Ile Ile Thr Thr Asp Arg Ile Lys Ser Glu Ser Asn Gly 945 950 955 960	2880
ccc cgg agg aga atg tta aga aga agg tta gat gct ttg tct aat caa Pro Arg Arg Arg Met Leu Arg Arg Leu Asp Ala Leu Ser Asn Gln 965 970 975	2928
ctt atc cag gtt att aat gaa ctt tat aat atc agt gac gaa gaa tat Leu Ile Gln Val Ile Asn Glu Leu Tyr Asn Ile Ser Asp Glu Glu Tyr 980 985 990	2976
acg aca gtt ttg aat gat gaa atg ttg aca gcg gcg tta gga gaa gaa Thr Thr Val Leu Asn Asp Glu Met Leu Thr Ala Ala Leu Gly Glu Glu 995 1000 1005	3024
aaa tga Lys 1010	3030

neb-183-cip.txt

<210> 4  
<211> 1009  
<212> PRT  
<213> Bacillus pumilus

<400> 4  
Met His Ile Ser Glu Leu Val Asp Lys Tyr Lys Ala His Arg Ser Thr  
1 5 10 15  
Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile  
20 25 30  
Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly  
35 40 45  
Lys Thr His Ile Leu Arg Asp Val Ile Gln Glu Glu Tyr Ile Glu Ile  
50 55 60  
Lys Asp Glu Glu Thr Lys Lys Asn Pro Asp Tyr Thr Leu Arg Ile Asn  
65 70 75 80  
Gly Thr Arg Lys Leu Phe Val Glu Val Lys Lys Pro Ser Phe Asn Ile  
85 90 95  
Leu Lys Ser Ala Lys Ala Ala Phe Gln Thr Arg Arg Tyr Gly Trp Ser  
100 105 110  
Ala Asn Leu Gly Ile Ser Val Leu Thr Asn Phe Glu His Leu Val Ile  
115 120 125  
Tyr Asp Cys Arg Tyr Thr Pro Asp Lys Ser Asp Asn Glu His Ile Ala  
130 135 140  
Arg Tyr Lys Val Phe Ser Tyr Glu Glu Tyr Glu Glu Ala Phe Asp Glu  
145 150 155 160  
Ile Lys Asp Ile Ile Ser Tyr Glu Ser Ala Asn Ser Gly Ala Leu Asp  
165 170 175  
Glu Met Phe Asp Val Asn Thr Arg Val Gly Glu Thr Phe Asp Glu Tyr  
180 185 190  
Phe Leu Gln Gln Ile Glu Asn Trp Arg Glu Lys Leu Ala Lys Thr Ala  
195 200 205  
Ile Lys Asn Asn Thr Glu Leu Gly Glu Glu Asp Val Asn Phe Ile Val  
210 215 220  
Gln Arg Leu Leu Asn Arg Ile Ile Phe Leu Arg Val Cys Glu Asp Arg  
225 230 235 240  
Thr Ile Glu Lys Tyr Glu Thr Ile Lys Ser Ile Lys Asn Tyr Glu Glu  
245 250 255  
Leu Lys Asp Leu Phe Gln Lys Ser Asp Arg Lys Phe Asn Ser Gly Leu  
260 265 270  
Phe Asp Phe Ile Asp Asp Thr Leu Leu Leu Glu Val Glu Ile Asp Ser  
275 280 285

neb-183-cip.txt

Asn Val Leu Ile Glu Ile Phe Ser Asp Leu Tyr Phe Pro Gln Ser Pro  
290 295 300

Tyr Asp Phe Ser Val Val Asp Pro Thr Ile Leu Ser Gln Ile Tyr Glu  
305 310 315 320

Arg Phe Leu Gly Gln Glu Ile Ile Ile Glu Ser Gly Gly Thr Phe His  
325 330 335

Ile Thr Glu Ser Pro Glu Val Ala Ala Ser Asn Gly Val Val Pro Thr  
340 345 350

Pro Lys Ile Ile Val Glu Gln Ile Val Lys Asp Thr Leu Thr Pro Leu  
355 360 365

Thr Glu Gly Lys Lys Phe Asn Glu Leu Cys Asn Leu Lys Ile Ala Asp  
370 375 380

Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Asp Phe Leu  
385 390 395 400

Val Glu Lys Val Met Glu Lys Ile Ile Glu Glu Asn Ile Asp Asp Ser  
405 410 415

Asp Leu Val Tyr Glu Thr Glu Glu Gly Leu Ile Leu Thr Leu Lys Ala  
420 425 430

Lys Arg Asn Ile Leu Glu Asn Asn Leu Phe Gly Val Asp Val Asn Pro  
435 440 445

Tyr Ala Val Glu Val Ala Glu Phe Ser Leu Leu Leu Lys Leu Leu Glu  
450 455 460

Gly Glu Asn Glu Ala Ser Val Asn Asn Phe Ile His His Glu Asp  
465 470 475 480

Lys Ile Leu Pro Asp Leu Thr Ser Ile Ile Lys Cys Gly Asn Ser Leu  
485 490 495

Val Asp Asn Lys Phe Phe Glu Phe Met Pro Glu Ser Leu Glu Asp Asp  
500 505 510

Glu Ile Leu Phe Lys Ala Asn Pro Phe Glu Trp Glu Glu Phe Pro  
515 520 525

Asp Ile Met Ala Asn Gly Gly Phe Asp Ala Ile Ile Gly Asn Pro Pro  
530 535 540

Tyr Val Arg Ile Gln Asn Met Lys Lys Tyr Ser Pro Glu Glu Ile Glu  
545 550 555 560

Tyr Tyr Gln Ser Lys Asp Ser Glu Tyr Thr Val Ala Lys Lys Glu Thr  
565 570 575

Val Asp Lys Tyr Phe Leu Phe Ile Glu Arg Ala Leu Ile Leu Leu Asn  
580 585 590

Pro Thr Gly Leu Leu Gly Tyr Ile Ile Pro His Lys Phe Phe Ile Thr  
595 600 605

Lys Gly Gly Lys Glu Leu Arg Lys Phe Ile Ala Glu Lys His Gln Ile  
610 615 620

## neb-183-cip.txt

Ser Lys Ile Ile Asn Phe Gly Val Thr Gln Val Phe Pro Gly Arg Ala  
 625 630 635 640  
 Thr Tyr Thr Ala Ile Leu Ile Ile Gln Ala Asn Lys Met Ala Gln Phe  
 645 650 655  
 Lys Tyr Lys Lys Val Ser Asn Ile Ser Ala Glu Thr Leu Asp Ser Glu  
 660 665 670  
 Glu Asn Thr Cys Val Tyr Ser Ser Glu Lys Tyr Asn Ser Asp Pro Trp  
 675 680 685  
 Ile Phe Leu Ser Pro Glu Thr Glu Ala Val Phe Thr Lys Phe Thr Glu  
 690 695 700  
 Ala Gln Phe Glu Lys Leu Gly Glu Ile Thr Asp Ile Ser Val Gly Leu  
 705 710 715 720  
 Gln Thr Ser Ala Asp Lys Ile Tyr Ile Phe Ile Pro Glu Asn Glu Thr  
 725 730 735  
 Ser Asp Thr Tyr Ile Phe Asn Tyr Lys Gly Lys Arg Tyr Glu Ile Glu  
 740 745 750  
 Lys Ser Ile Cys Cys Pro Ala Ile Tyr Asp Leu Ser Phe Gly Ser Phe  
 755 760 765  
 Glu Ser Ile Gln Gly Asn Ala Gln Met Ile Phe Pro Tyr Glu Ile Arg  
 770 775 780  
 Asp Glu Glu Ala Tyr Leu Leu Glu Glu Glu Thr Leu Glu Asn Asp Tyr  
 785 790 795 800  
 Pro Leu Ala Trp Asn Tyr Leu Asn Glu Phe Lys Glu Ala Leu Glu Lys  
 805 810 815  
 Arg Ser Leu Gln Gly Arg Asn Pro Lys Trp Tyr Gln Tyr Gly Arg Ser  
 820 825 830  
 Gln Ser Leu Ser Lys Phe His Asp Lys Glu Lys Leu Ile Trp Thr Val  
 835 840 845  
 Leu Ala Thr Lys Pro Pro Tyr Val Leu Asp Arg Asn Asn Leu Leu Phe  
 850 855 860  
 Thr Gly Gly Gly Asn Gly Pro Tyr Tyr Gly Leu Ile Asn Gln Ser Ile  
 865 870 875 880  
 Tyr Ser Leu His Tyr Phe Leu Gly Ile Leu Ser His Pro Val Ile Glu  
 885 890 895  
 Ser Met Val Lys Ala Arg Ala Ser Glu Phe Arg Gly Ser Tyr Tyr Ser  
 900 905 910  
 His Gly Lys Gln Phe Ile Glu Lys Ile Pro Ile Arg Lys Ile Asp Phe  
 915 920 925  
 Asp Asp Gln Asp Glu Val Asp Lys Tyr Asn Thr Val Val Thr Thr Val  
 930 935 940  
 Glu Lys Leu Ile Ile Thr Thr Asp Arg Ile Lys Ser Glu Ser Asn Gly  
 945 950 955 960

neb-183-cip.txt

Pro Arg Arg Arg Met Leu Arg Arg Arg Leu Asp Ala Leu Ser Asn Gln  
965 970 975

Leu Ile Gln Val Ile Asn Glu Leu Tyr Asn Ile Ser Asp Glu Glu Tyr  
980 985 990

Thr Thr Val Leu Asn Asp Glu Met Leu Thr Ala Ala Leu Gly Glu Glu  
995 1000 1005

Lys

<210> 5  
<211> 24  
<212> DNA  
<213> *Bacillus pumilus*

<400> 5  
gtggaaacgg accgtattat ggtt

24

<210> 6  
<211> 24  
<212> DNA  
<213> *Bacillus pumilus*

<400> 6  
caccagtaaa taacaggta ttcc

24

<210> 7  
<211> 27  
<212> DNA  
<213> *Bacillus pumilus*

<400> 7  
ttcgtaccaa gtacggtcca tatcagt

27

<210> 8  
<211> 27  
<212> DNA  
<213> *Bacillus pumilus*

<400> 8  
ccgtatgtac ttgataggaa taacctg

27

<210> 9  
<211> 24  
<212> DNA  
<213> *Bacillus pumilus*

<400> 9  
aggaactaag aaagttcata gctg

24

<210> 10  
<211> 24  
<212> DNA

neb-183-cip.txt

<213> Bacillus pumilus		
<400> 10 atgcggattt atataaccca acag	24	
<210> 11		
<211> 24		
<212> DNA		
<213> Bacillus pumilus		
<400> 11 tgacgtcctc ttcaccta atcg	24	
<210> 12		
<211> 24		
<212> DNA		
<213> Bacillus pumilus		
<400> 12 gagtttgtga agatagaacc attg	24	
<210> 13		
<211> 48		
<212> DNA		
<213> Bacillus pumilus		
<400> 13 agcggatccg gaggtaaata aatgaatcaa ttaattgaaa atgttaat	48	
<210> 14		
<211> 42		
<212> DNA		
<213> Bacillus pumilus		
<400> 14 aaggggcat gcttataactt atttcttcgt tctattgtt ct	42	
<210> 15		
<211> 51		
<212> DNA		
<213> Bacillus pumilus		
<400> 15 caaggatccg gaggtaaata aatgcataata agtgagttag tagataaata c	51	
<210> 16		
<211> 36		
<212> DNA		
<213> Bacillus pumilus		
<400> 16 ttaggatcct cattttctt ctcctaacgc cgctgt	36	

neb-183-cip.txt

<210> 17  
<211> 54  
<212> DNA  
<213> *Bacillus pumilus*

<400> 17  
caccaatcta gaggaggtaa ataaatgcat ataagtgagt tagtagataa atac 54

<210> 18  
<211> 42  
<212> DNA  
<213> *Bacillus pumilus*

<400> 18  
tgaaatctcg agttatcctg atccacaaca tatatatgct at 42

<210> 19  
<211> 54  
<212> DNA  
<213> unknown

<220>  
<223> Synthetic primer

<400> 19  
caccaatcta gaggaggtaa ataaatgcat ataagtgagt tagtagataa atac 54

<210> 20  
<211> 39  
<212> DNA  
<213> unknown

<220>  
<223> Synthetic primer

<400> 20  
gtttatacga agtgtataag ctggattttt ctttgtctc 39

<210> 21  
<211> 39  
<212> DNA  
<213> unknown

<220>  
<223> Synthetic primer

<400> 21  
gagacaaaga aaaatccagc ttatacactt cgtataaac 39

<210> 22  
<211> 36  
<212> DNA  
<213> unknown

<220>  
<223> Synthetic primer

<400> 22

neb-183-cip.txt

ttaggatcct catttttctt ctccctaacgc cgctgt	36
<210> 23	
<211> 54	
<212> DNA	
<213> unknown	
<220>	
<223> Synthetic primer	
<400> 23	
ggtgtttcta gaggaggtaa ataaatgtct aatgaaaatt ataacattga tttc	54
<210> 24	
<211> 39	
<212> DNA	
<213> unknown	
<220>	
<223> Synthetic primer	
<400> 24	
ggtggtgagc tcctattgac ataatcgatc atcaagaag	39
<210> 25	
<211> 42	
<212> DNA	
<213> unknown	
<220>	
<223> Synthetic primer	
<400> 25	
atagggtgga ttgcctaata ttacatcaa gccaccattt gc	42
<210> 26	
<211> 40	
<212> DNA	
<213> unknown	
<220>	
<223> Synthetic primer	
<400> 26	
tttgatgtaa tattaggcaa tccaccctat ataagaattc	40